TRICHOPHYTON MENTAGROPHYTES (VARIETY NODULAR)

A Mutant with Brilliant Orange-Red Pigment Isolated in Nine Cases of Ringworm of the Skin and Nails*,**

LUCILLE K. GEORG, Ph.D. and EUGENIA H. MAECHLING, Ph.D.

During the past two years at the Vanderbilt Clinic, New York City, we have obtained from patients presenting superficial lesions of the glabrous skin and nails, four fungus cultures showing brilliant orange-red pigment which were grossly and microscopically unlike the usual dermatophyte species. During this same period we received for identification five similar cultures isolated from patients in various parts of the United States and Canada. The unusual appearance of the cultures, as well as the lack of certain structures in many of the isolates, made it difficult to classify them among the known species of fungi. However, an extensive study of these strains as well as single spore analyses from a rapidly mutating culture led us to conclude that they are mutants derived from Trichophyton mentagrophytes, a common agent of ringworm of the glabrous skin and nails. The fact that nine similar cultures have now been isolated from patients in various parts of the country, indicates that this form should be described so that it may be identified as it appears in the future.

The sources of the cultures were as follows: two strains from Iowa City, Iowa (Dr. Evelyn Wallace and Dr. R. G. Carney); one strain isolated in Philadelphia, Pennsylvania (Mr. Gerbert Rebell); two strains from Toronto, Canada (Mr. J. B. Fischer); and four strains isolated in the Mycology Laboratory of Columbia University, College of Physicians and Surgeons, New York City. All strains were obtained from lesions of the glabrous skin and nails which appeared typical of ringworm produced by the common dermatophytes. The strains appear to be identical with the exception of one which shows no spores and is apparently completely pleomorphic. The inclusion of this strain is on the basis of the brilliant orange-red pigment only. A description of a typical sporing strain follows.

Sabouraud's dextrose agar (*Difco*). *1 week:* The colony is flat and has a diameter of 10–15 mm. The surface is covered with a moderately heavy white, downy to fluffy growth. At the edge of the colony where the surface growth is less dense can be seen the brilliant yellow-orange pigment which is clearly evident on the reverse side of the colony (Ridgeway (1), "Orange chrome," Plate II, color ✻11), (fig. 1).

3 weeks: In the center of the colony the aerial mycelium develops a light yellow to buff pigment. The periphery of the colony is now usually surrounded by a halo of submerged mycelial growth in irregularly branched fern-like strands

* From the Department of Dermatology, College of Physicians and Surgeons, Columbia University, New York City.

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which are brilliant yellow to orange. The reverse side of the colony now shows a deep orange-red center (Ridgeway (1), "Scarlet-red," Plate II, color ⅋3) which

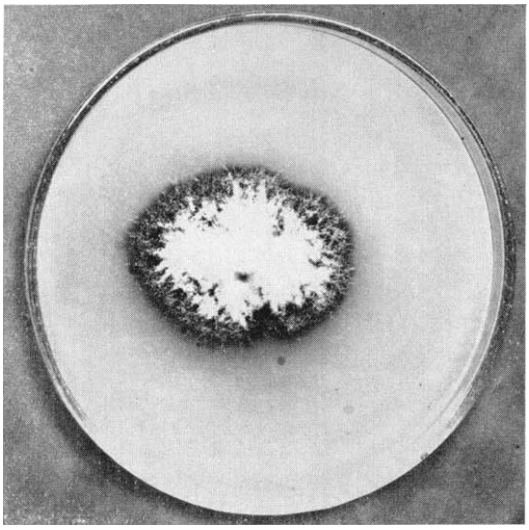


FIG. 1. Trichophyton mentagrophytes (var.: nodular), Sabouraud's dextrose agar—1 week.

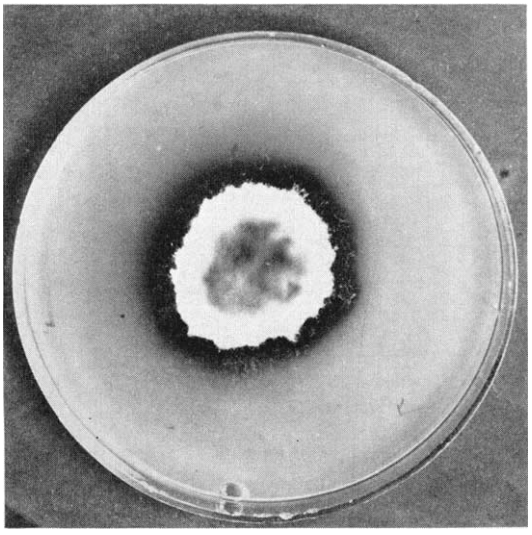


FIG. 2. Trichophyton mentagrophytes (var.: nodular), Sabouraud's dextrose agar—3 weeks.

fuses irregularly into the orange and yellow pigments. Frequently a deep orange-red veining extends to the edge of the colony and into the peripheral subsurface growth (fig. 2).

6 weeks: The colony fills the entire Petri dish or agar slant and the aerial mycelium which now covers the entire colony has become a deep rose-pink. The

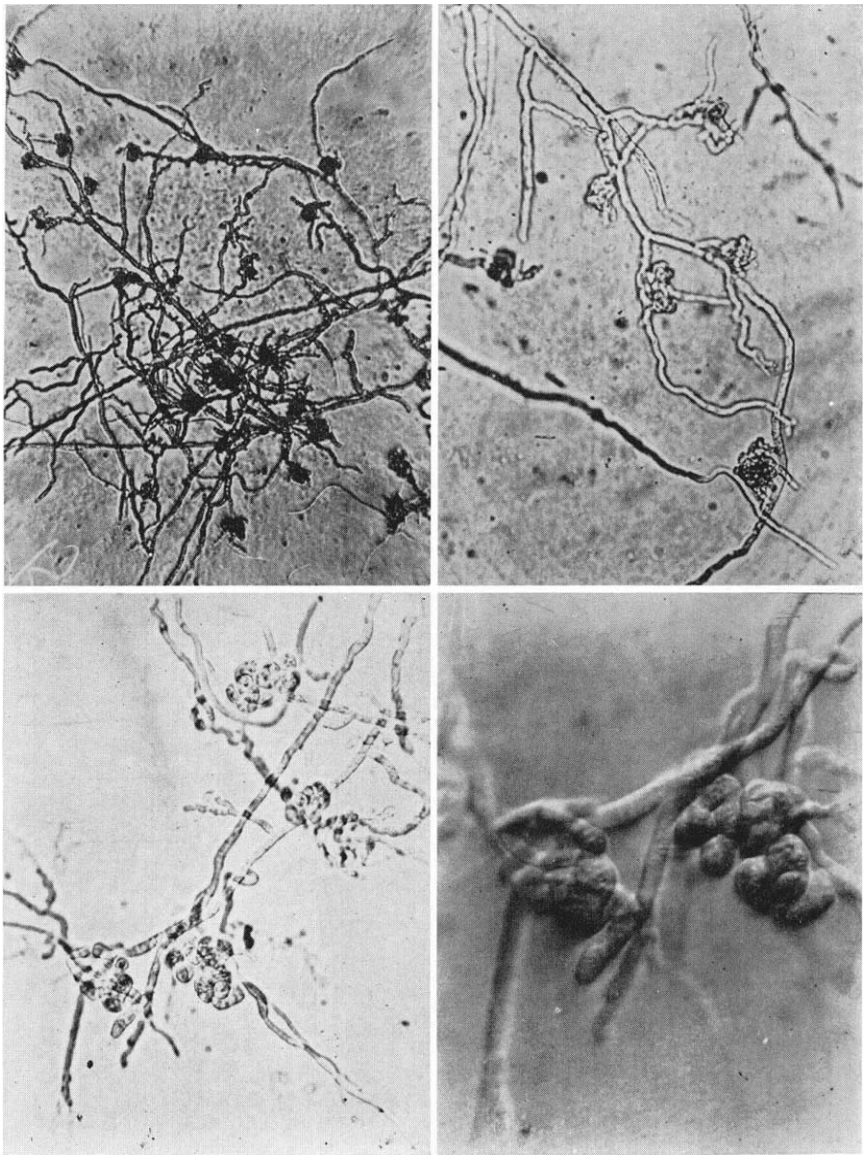


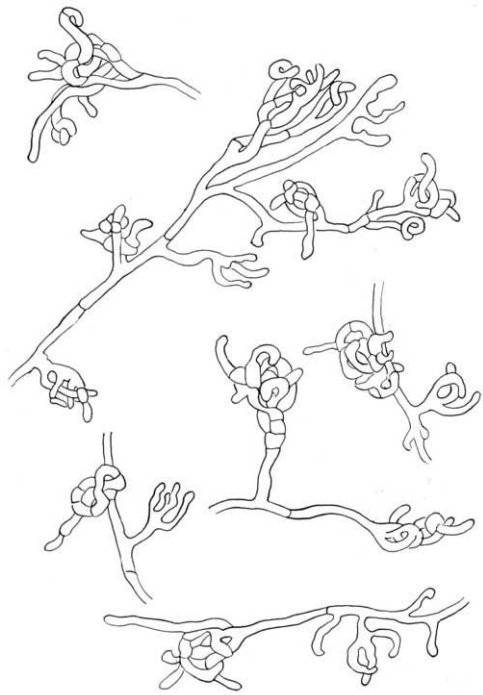
FIG. 3 (a, b, c, and d). Trichophyton mentagrophytes (var.: nodular), cornmeal agar—microscopic morphology showing nodular bodies (160×, 400×, 600×, 800×).

reverse side of the colony is now a very dark red, becoming brownish-mahogany in the center.

The change in pigment from the brilliant yellow in the young colonies to the deep orange-red as the colony ages is due to the production of an alkaline medium.

The pigment which may be extracted with water and alcohol, changes from yellow to orange-red as alkali is added.

Microscopic examinations of the growth on Sabouraud's dextrose agar revealed a fairly regular, branching, septate mycelium of normal diameter for the dermatophytes. Many microconidia were found in the aerial mycelium borne in clusters (en grappe) on the ends of mycelia, as well as scattered irregularly along the sides of the hyphae (en thyrse). These are small, globose to subglobose, and occasionally elongate. No spirals or macroconidia were observed in freshly isolated



Trichophyton mentagrophytes (var. nodular)
submerged mycelium — cornmeal agar 800X

strains. The sub-surface mycelium is usually thicker than the aerial mycelium and is characteristically very twisted and highly branched. At fairly regular intervals, clusters of short twisted side branches occur. Frequently a single side branch will show secondary divisions a short distance from its origin into three or four irregular short branches. The short side branches in each group tend to twist together to form a nodular body usually with one or two ends projecting from the mass. These nodular bodies were found in large numbers in all of the strains studied and were the most striking characteristic seen on microscopic examination. Some of the heavier strands of submerged mycelium, as well as the nodular bodies, contain deep yellow pigment (fig. 3, a, b, c, and d) (also camera lucida drawings).

Cornmeal-dextrose agar (prepared with chemically pure (C.P.) dextrose). On this medium young cultures show a scant aerial mycelium with many conidia as described above. Submerged growth shows large numbers of nodular bodies scattered regularly throughout the entire growth. These may appear by the end of four to six days. Some bright yellow pigment may appear at the point of the inoculation, but the red pigment does not develop on this medium.

Wort agar (*Difco*). The growth is similar to that on cornmeal-dextrose agar. Conidia are numerous in the aerial mycelium. Submerged growth shows large numbers of nodular bodies. The pigment on the reverse side of the colony is a deep red-brown. Pleomorphic tufts occur quite frequently on this medium.

EXPERIMENTAL STUDIES

One of the strains of the orange-red cultures was of particular interest because it had appeared as one of many satellite colonies about a white, granular colony which was both grossly and microscopically a typical Trichophyton mentagrophytes. This culture appeared to be mutating and the scattered spores were producing a new type of growth. To determine whether this was the case, a spore suspension was made from the white, granular colony which we shall designate as T. mentagrophytes (normal variety) and a series of 30 spores were isolated for monospore cultures (2). All of the 30 cultures derived resembled the parent culture and may be described on Sabouraud's dextrose-agar as follows: A chalky-white, flat, disc-shaped, granular colony with light yellow to buff color developing in the center. The pigment on the reverse side of the colony is a light yellow to tan, later becoming a light rose-brown (fig. 4).

The granular surface is filled with microconidia, globose and subglobose, many in heavy clusters (en grappe) and also occurring along the mycelium (en thyrse). Tightly wound spirals occurred in most transplants, especially on cornmeal agar. Macroconidia were numerous. These were smooth-walled, clavate and from 3 to 5 segmented; many very long ones were observed. Macroconidia were formed in great numbers on wort agar. No nodular bodies were found on any medium. On cornmeal-dextrose agar (prepared with C.P. dextrose) only a light yellowish pigment was produced. Figure 5 shows the characteristic microscopic morphology. It appeared evident that these cultures were typical colonies of T. mentagrophytes.

Most of these cultures remained stable for several weeks. A few, however, began to develop bright yellow streaks on their reverse sides. One culture, No. 7, was selected as a stable normal variety and a series of spores were isolated from it. In a series of 28 monospore cultures derived from this monospore strain, two were variant cultures. One designated as Spore P was a brilliant orange-red culture similar in all respects to the orange-red cultures studied in this series and described above. The aerial mycelium showed large numbers of conidia and the submerged mycelium was filled with the characteristic nodular bodies.

The second variant culture, Spore Y, produced yellow and orange pigments only during the first few days of its growth, the pigment changing very quickly to dark red. By the end of the first week the entire back of the colony was a deep

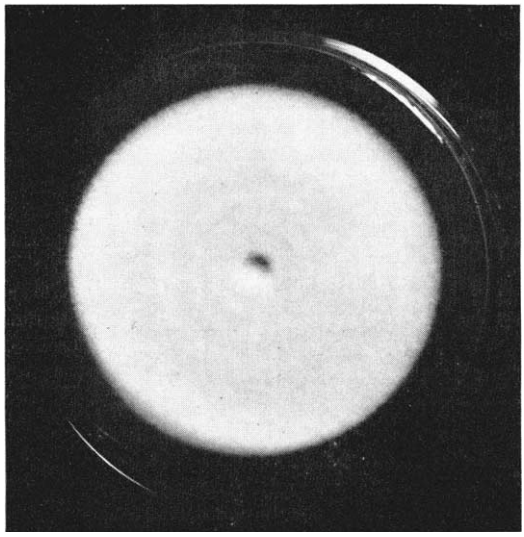


FIG. 4. Trichophyton mentagrophytes. Monospore culture #7 (normal variety), Sabouraud's dextrose agar—3 weeks.

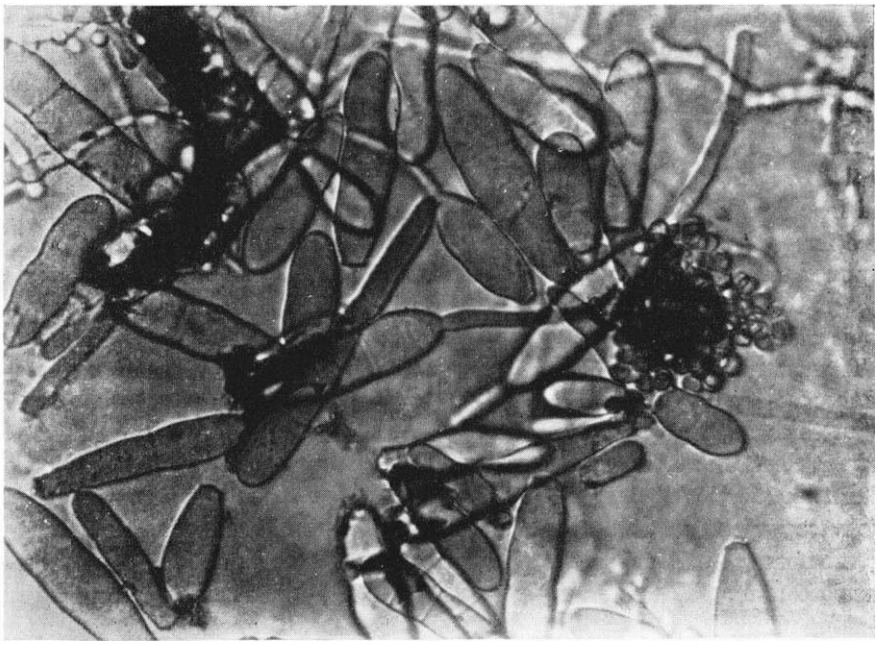


FIG. 5. Trichophyton mentagrophytes. Monospore culture #7 (normal variety), Wort agar—2 weeks. Microscopic morphology showing characteristic macroconidia and clusters of microconidia.

red. The microscopic morphology was similar in all respects to that of the orange-red cultures except that in one instance on wort agar a few macroconidia were produced which resembled those of the parent (normal variety) culture.

Thus the orange-red, nodular variety was found to have developed spontaneously from a single spore derived from a monospore culture of Trichophyton mentagrophytes. Spore suspensions of this culture stained by the Feulgen technic (according to the method of DeLamater (3)) indicated that the conidia were uninucleate, and thus it seems apparent that a genetic change or mutation must have occurred in the production of these variants.

The Spore P and Spore Y mutants as well as the other orange-red cultures in this series have remained stable over a period of two years and, except for the fact that several of the strains have on occasion produced spirals in large numbers, they have not reverted to the parent (normal variety) culture. Several, however, have produced pleomorphic tufts, and two of the cultures have at this time become completely pleomorphic; that is, they produce a rapidly growing, woolly, aerial mycelium which is sterile. Once the culture has become pleomorphic, it no longer shows the characteristic nodular bodies, and only the bright orange pigment on the back of the colony suggests its original appearance.

A study was made of the orange-red pigment in the mutant colonies in order to determine its chemical nature. The light cream to rose pigment of the normal variety of T. mentagrophytes was also studied in view of the relationship of this commonly seen form to the brilliantly pigmented mutants.

The method of extraction and chemical studies of these pigments will be described fully in another paper. The results indicated that the orange-red pigment isolated from the mutant strains as well as the small amount of yellowish pigment from the normal variety were chemically identical. The intense pigmentation of the mutant strains therefore appeared to be due to a large increase in the amount of the same pigment produced in the normal variety. The pigment was chemically similar to pigments produced by various species of trichophytons which have been described by Tate (4) as "of the nature of anthracene pigments." Tate pointed out that the red colors of the alkaline solutions could be changed by reduction with sodium hydrosulphite to a faint yellow and reoxidized by contact with air, the color change being reversible. These color changes by reduction and reoxidation in alkaline solution, also observed with the pigments extracted from the T. mentagrophytes strains studied here, are a characteristic property of anthraquinone and its derivatives.

The pigments of the cultures of the normal variety and the orange-red, nodular variety of T. mentagrophytes were found to be completely confined within the cells of the mycelium. The fungi were grown on Sabouraud's dextrose agar slants, the mycelial mat was separated from the medium, and ground in a mortar with glass powder in order to break up the mycelium. It was necessary to further treat the ground mycelium with glacial acetic or mineral acids before extracting the pigment with chloroform or other organic solvents. It seems possible that the pigments are present as glucosides, as polyhydroxy-anthraquinones frequently occur in this form in certain plants.

It seems of interest to note that the pigments of the plant pathogen Helminthosporium gramineum (Rabenhorst), described by Charles, Raistrick, Robinson and Todd (5), were anthraquinone derivatives which could be extracted from the powdered mycelium directly with chloroform. These investigators stated that a microscopic examination of the mycelium revealed well formed, very dark red, needle-shaped crystals.

ANIMAL INOCULATIONS

The monospore cultures, (1) the white, granular (normal variety) derived from Spore #7, (2) the orange-red strain from Spore P, and (3) the predominantly red strain from Spore Y were used in an attempt to produce lesions in rabbits. The animals were inoculated by rubbing their shaved, scarified sides with a spore mass scraped with a sterile scalpel from the surface of week-old colonies on Sabouraud's dextrose agar plates. On the sixth or seventh day some redness and scaling was visible on all of the rabbits. Scrapings from these areas showed branching mycelia in the skin and cultures developed from this material resembled in all respects the original cultures used for inoculation. Thus the normal variety, the orange-red and red nodular varieties were shown to remain stable through animal passage.

DISCUSSION

Trichophyton mentagrophytes, the type species of the "gypseum group," is commonly described on Sabouraud's dextrose agar as having a white to tan, granular to downy, flat, spreading colony with light tan to rose-brown pigmentation on the reverse side. A very wide range of variation, however, occurs in this group, colonies with various pigments and textures being included. A great number of species have been described, and a vast amount of literature has resulted from studies of these organisms. However, many workers have noted the spontaneous occurrence of variations in laboratory cultures with the production of new types of growth which differed as much from each other and the parent cultures as do many of the described species in the group. This suggests that these organisms are highly mutable and that many variations in gross and microscopic appearance of the cultures should be expected. It has been suggested by those who have studied variations in the "gypseum group" of trichophytons, that many of the so-called "species" should not have specific designation, but should be considered variants of the type species which is now known as Trichophyton mentagrophytes.

Variations are most likely to occur in old laboratory cultures and frequently appear as spots or sectors of aberrant growth. These variations are of two types: the first, called pleomorphism (woolly degeneration), refers to the production of rapidly growing, white, fluffy areas consisting of thin, sterile mycelium. This variant is considered stable and is not known to revert to the original form. A colony completely covered with this type of growth resembles the "species" described as Trichophyton interdigitale. A culture of such appearance may occasionally be isolated directly from a lesion. If no spores (microconidia or macroconidia) can be found, identification of the culture may be impossible. However,

usually a few microconidia are present in the culture, and by repeated transplantations, areas of spore-bearing growth may become evident and can be selected for subculture.

The second type of variation refers to the spontaneous production of spore-bearing growth which, when subcultured, produces colonies that differ from the parent culture in form, texture, pigmentation, or microscopic morphology. These variants may remain stable, may revert to the type of the parent culture, or may eventually change to the pleomorphic type of growth with woolly, sterile mycelium. That such variations can occur spontaneously in cultures derived from a single spore (conidium) was shown by Emmons (6) in 1932 in a study of monospore strains of Trichophyton mentagrophytes. If it is assumed that each conidium contained but a single nucleus, such a variation would indicate that a genetic change or mutation had occurred. In a later study, Emmons and Hollaender (7) demonstrated that many of the mutations which occurred spontaneously in the laboratory could be induced by the ultraviolet irradiation of spore suspensions. They were able to induce the production of mutant cultures which varied so widely from the original parent culture that, had the origin been unknown, many could not have been identified. Several of the mutants resembled other of the so-called "species" of the gypseum group: T. interdigitale, T. niveum, T. granulosum.

As the result of studies described above with a single spore strain of a typical Trichophyton mentagrophytes (i.e., a culture which closely resembles, both grossly and microscopically, the descriptions given for the type species of the "gypseum group"), we may consider the orange-red cultures also as mutants of Trichophyton mentagrophytes. In order to avoid the addition of further "species" to this highly variable group we suggest that these mutants be considered as variants and "variety nodular" is used as descriptive of the characteristic microscopic appearance.

Nodular bodies have been reported in many of the dermatophytes; they occur frequently in trichophytons of the "gypseum group." Sabouraud (8) described several strains which produce them with regularity. One of these, Trichophyton lacticolor, develops them in large numbers, especially in the thin peripheral growth about the colonies. They appear particularly in areas which are deeply pigmented, "a canary yellow which becomes very deep on glucose agar." He describes these nodular bodies as developing from a row of cells which resemble a young macroconidium; the cells elongate, curve, and coil until a mass of double-walled chlamydospores are formed. We have studied a culture of T. lacticolor obtained from the Sabouraud collection and have observed that the nodular bodies which are quite numerous do frequently form in this manner. They also form by a simple coiling of adjacent mycelial branches, or may form complex masses from the intertwining of many mycelial branches. Biltris (9) observed large numbers of nodular bodies in his strain R.B.S., a red strain derived from a culture of Trichophyton gypseum (asteroides). Emmons (6) has described a variant (O) arising spontaneously from a single spore culture of Trichophyton gypseum which showed many clumps "suggestive of nodular organs," and a variant (W) resembling Trichophyton gypseum (lacticolor) which showed large

numbers of nodular bodies. Emmons (10) states that the nodular organ is the most interesting structure formed by the dermatophytes, and one which gives the strongest evidence of a relationship with the ascomycetes. It bears a strong resemblance to the ascogonium seen in many of the lower ascomycetes; however, it never develops into a fertile ascocarp.

Brilliant yellow to orange and red pigments are also not uncommon in the "gypseum group" of trichophytons. Biltris (9) reported a red strain (R.B.S., mentioned above) which was the color of a "half-ripe strawberry," and a variant from this strain produced colonies surrounded by a fern-like growth of submerged mycelium which was canary yellow in color. Subcultures from the yellow areas produced small yellow-vermicellular colonies which became red on the reverse side at the end of the fourth week. It is interesting that this culture later developed a segment resembling Trichophyton lacticolor and presented large numbers of "beautiful nodular organs." Biltris also demonstrated that this yellow pigment would turn red if treated with ammoniacal alcohol. Catanei (11), in describing the changes which occur during the course of aging of a culture of Trichophyton gypseum, describes the development of a "yellow type" that starts as a small glabrous knob "yellow as gold—sometimes orange" from which short rays of growth extend into the medium, and these are equally pigmented. He considered that the "yellow type" represented a stage in the degradation of the colony, as there were fewer conidia than in the original culture and a concomitant increase in pigmented mycelia and chlamydospores. This "yellow type," however, was not stable and by animal passage returned to the original type. Emmons and Hollaender (7) have been able to produce several golden pigmented variants of Trichophyton mentagrophytes (asteroides) by irradiating a spore suspension with ultraviolet light.

We very commonly isolate strains of T. mentagrophytes which develop a dark red pigment on the reverse side of the colony after one to three weeks on the usual Sabouraud's medium. Even the aerial mycelium may become pink as the culture ages. At times the pigment is as intense as the wine-red of T. rubrum, and differentiation from this species must be made on the basis of the microscopic morphology, especially the characteristic macroconidium. A method for promoting the production of the typical macroconidium of T. rubrum by cultivation on heart infusion agar has been described by Benham (12), and this aids in the differentiation of these "red strains." Another method for separating the red stains of T. mentagrophytes from T. rubrum, described by Bocobo and Benham (13), makes use of a one percent dextrose-cornmeal agar prepared with chemically pure (C.P.) dextrose. On such a medium, the rubrum cultures will produce their characteristic wine-red pigment, but the "red mentagrophytes" cultures produce only a light yellow to tan pigment.

SUMMARY

1. Nine strains of an orange-red variant of Trichophyton mentagrophytes have been isolated from ringworm of the glabrous skin and nails during the past two years.

2. The cultures, obtained from various parts of the United States, appear to be identical and vary from the usually isolated "normal type" T. mentagrophytes in the following characteristics:

(a) A brilliant yellow pigment is produced by the young colony. As growth continues, and the medium becomes alkaline, the reverse side of the colony becomes a brilliant orange-red. The color is deeper in the center and in radiating strands of the heavier growth.

(b) The aerial mycelium is more fluffy and grows more rapidly than that of the "normal type." However, it tends to be sparse at the periphery of the colony where fern-like, bright yellow to orange-red, submerged growth is usually visible.

(c) The aerial mycelium contains fewer conidia than the "normal type."

(d) Spirals and macroconidia are rarely seen.

(e) The submerged mycelium is filled with large numbers of characteristic nodular bodies—described in the text.

3. By the study of single spore isolates from a monospore culture of the "normal type" mentagrophytes, variants have been obtained which resemble the orange-red cultures described in this series. Since the conidia of T. mentagrophytes have been shown to be uninucleate, these orange-red cultures are considered as true mutants.

4. The orange-red pigment of the mutant forms was found to be chemically identical to the cream to rose pigment of the normal strain of T. mentagrophytes; the striking change in color between the normal and nodular varieties of T. mentagrophytes being of a quantitative nature. This pigment, which is confined within the mycelial cells, is an anthraquinone derivative similar to those described in other dermatophytes, and appears to be present as a glucoside.

5. The mutants have been stable for over two years on Sabouraud's dextrose agar, and remain stable through animal passage.

6. These mutants are designated as Trichophyton mentagrophytes (variety: nodular).

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